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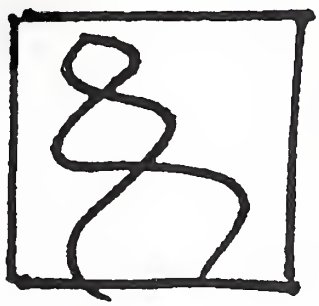



## *TRANSGENIC FISH RESEARCH: UPDATE*
## January 1993 - March 1995

Prepared September 28, 1995
for the USDA Office of Agricultural Biotechnology

1

**All-fish gene constructs for growth hormone gene transfer in fish. carp transgenic fish construction by expression of gilthead seabream somatotropin gene (conference paper)**

Cavari-B; Hong-Y; Funkenstein-B; Moav-B; Schartl-M *Fish-Physiol.Biochem.; (1993) 11, 1-6, 345-52* (1993).

*Descriptor:* carp transgenic fish construction, gilthead seabream somatotropin gene cloning, vector plasmid ptMTa-gbsGHcDNA, plasmid ptMTb-gsbGHcDNA, plasmid pcA-beta-gsbGHcDNA, inducible metallothionein promoter, expression in carp epithelium cell culture * hormone Sparus aurata gene transmission

*Abstract*: In order to prepare transgenic fish which grow to a large size, the following constructs were prepared: (1) a rainbow trout metallothionein a/b and the gilthead seabream (Sparus aurata) somatotropin (GH) cDNA (plasmid ptMTa-gbsGHcDNA, plasmid ptMTb-gsbGHcDNA), and carp beta-actin S. aurata GH cDNA (plasmid pcA-beta-gsbGHcDNA). The inducible metallothionein promoters a (tMTa) (430 bp) and b (tMTb) (260 bp) were cloned from rainbow trout, and the constitutive beta-actin promoter was isolated from carp and cloned in plasmid pGEM- 3Z containing the GH cDNA of S. aurata to form plasmid ptMTa-gsbGH and plasmid ptMTb-gsbGH. The carp cytoplasmic beta-actin gene was used to isolate constitutive regulatory sequences. A regulatory sequence in plasmid pUC118 was ligated to GH cDNA of S. aurata to form plasmid pcA-beta-gsbGHcDNA. Expression of the constructs was tested in carp epithelium cell culture in Dulbecco's modified Eagle's medium with 10% fetal cattle serum and found to be induced by zinc. The ptMTagsb-GH cDNA construct was microinjected into fertilized carp eggs. About 25% of microinjected fish had GH cDNA integrated into their genome. (15 ref).

2

**Biotechnological applications of research on animal pigmentation. application of pigment gene cloning in transgenic animal breeding**

Hudon-J *Biotechnol.Adv.; (1994) 12, 1, 49-69* (1994).

*Descriptor:* pigment gene cloning, animal breeding, transgenic animal construction

*Abstract*: The implications of primary research on pigmentation for the color manipulation of animal species of economic importance, and the facilitation of specific processes in biotechnology are discussed. Pigment technologists, especially fowl and fish nutritionists, are concerned with achieving the often specific type and degree of coloration demanded by consumers of various products (notably egg yolk, eggshell, broiler skin and salmon flesh). In most instances involving melanin and porphyrin pigments, the desired coloration is achieved through the use of alternate alleles at gene loci controlling the characters of interest. The cloning of genes involved in pigmentation offers the prospect of deciphering the genetic control of animal pigmentation and modifying it to meet specific pigmentation needs. With the advent of molecular biology, the cloning of pigmentation genes and the ability to produce transgenics, there are strong desires to develop strains that can be sexed readily by means of a visible phenotype. (115 ref).

3

**Biotechnology in aquaculture, with special reference to transgenic salmon. salmon transgenic fish; a review**

Male-R; Lorens-J-B; Nerland-A-H; +Slinde-E *Biotechnol.Genet.Eng.Rev.; (1993) 11, 31-56* (1993).

*Descriptor:* salmon transgenic fish, aquaculture, review * transgenic animal

*Abstract*: The application of biotechnology in aquaculture, particularly with reference to transgenic salmon is reviewed. The following topics are.

4

**Characterization of a cell line derived from zebrafish (Brachydanio rerio) embryos. ZF4 cell culture characterization, transfection; potential use for gene regulation studies, transgenic fish construction, etc.**

Driever-W; Rangini-Z *In-Vitro; (1993) 29, 9, 749-54* (1993).

*Descriptor:* zebrafish cell culture line ZF4 characterization, transfection, pot. gene regulation studies, transgenic fish breeding

*Abstract*: Several cell lines were obtained from 1-day-old embryos of zebrafish (Brachydanio rerio). Embryos were mechanically dissociated and transferred to 48-well plates. After 24-48 hr, cells

started to migrate from the embryo fragments, and most wells became confluent with various cell types after 1 wk. Only colonies of fibroblast-like cells became established, of which line ZF4 had good growth characteristics and studied further. ZF4 was well adapted to growth in standard media supplemented with fetal cattle serum and was maintained for over 150 population doublings. The cell lines had a hyperploid karyotype. Transient transfection of the cells was achieved using the calcium phosphate coprecipitation method. Introduction of plasmids carrying the neomycin-resistance gene allowed selection of transformed cell lines with G418. Various promoter/enhancer systems used in studies in mammalian systems were active in ZF4 cells. The cell line will be useful for studies of gene regulation, for the introduction of DNA into the zebrafish germ line and transgenic fish breeding, and for studies of transposon-mediated insertional mutagenesis. (48 ref).

5

**Cloning and expression of flatfish (Paralichthys olivaceus) interferon cDNA. immortalized leukocyte HL-8 cell culture interferon gene expression in BHK-21 cell culture transfected with vector plasmid pSR-alpha- IFN; DNA sequence**

Tamai-T; +Shirahata-S; Noguchi-T; Sato-N; Kimura-S; Murakami-H *Biochim.Biophys.Acta-N; (1993) 1174, 2, 182-86* (1993).

*Descriptor:* flatfish immortalized leukocyte HL-8 cell recombinant interferon prep., gene cloning, DNA sequence, vector plasmid pSR-alpha-IFN expression in BHK-21 cell culture * Paralichthys olivaceus fish baby hamster kidney BHK mammal protein sequence virucide antitumor immunostimulant gene transmission

*Abstract*: Fish interferon (IFN) cDNA was cloned from a gene bank of immortalized flatfish (Paralichthys olivaceus) leukocyte cell line HL-8 (established by cotransfecting human c-Ha-ras and human c-fos) in plasmid pBluescript in Escherichia coli XL1 blue. The clone contained an open reading frame that encoded a 138 amino acid protein including a glycosylation site and a signal peptide containing 30 amino acids. The IFN cDNA was cloned into the EcoRI-PstI site of mammalian expression vector plasmid pcDL-SR-alpha-296 to form plasmid pSR-alpha-IFN. BHK-21 cells transfected with the IFN-expression vector plasmid pSR-alpha-IFN and cultured in ERDF medium with 5% fetal cattle serum at 37 deg under a 5% CO2 atmosphere produced recombinant IFN (mol.wt. about 16,000), which was then purified by WGA agarose affinity chromatography. The recombinant IFN prevented infection of carp epithelium and Chinook salmon embryo cells incubated with Hirame (flatfish) rhabdo virus (m.o.i. of 50). The IFN cDNA and its encoded protein were sequenced. The flatfish IFN may be useful for protection of various types of fish from infection by a range of fish viruses. (22 ref).

6

**Development of a heavy metal-inducible fish-specific expression vector for gene transfer in vitro and in vivo. rainbow trout metallothionein-B gene promoter isolation and use in vector construction for potential use in transgenic fish breeding (conference paper)**

Hong-Y; Winkler-C; Brem-G; +Schartl-M *Aquaculture; (1993) 111, 1-4, 215-26* (1993).

*Descriptor:* rainbow trout metallothionein-B promoter isol., expression in human, fish cell culture, pot. transgenic fish breeding, inducible, tissue- specific gene expression * Oncorhynchus mykiss vector

*Abstract*: The promoter of the rainbow trout (Oncorhynchus mykiss) metallothionein-B gene was isolated from genomic DNA by the polymerase chain reaction, fused to the bacterial chloramphenicol-acetyltransferase (EC-2.3.1.28) gene in expression vector plasmid pUC18 to generate plasmid pMTbCAT (4.6 kb), and functionally analyzed in HepG2 human hepatoblastoma cell culture, and Xiphophorus xiphidium A2 embryonal epitheloid, Xiphophorus PSM melanoma, carp EPC epithelioma populosum and rainbow trout hepatoma RTH-19 fish cells. The promoter exhibited an extremely low basal expression in all cell lines and was zinc-and cadmium-inducible except in the PSM cell line, where it was completely inactive. The metal-induced expression patterns were cell line-specific. In general, the fish promoter was more species-and cell type-specific than its human counterpart. In a transient assay, it was functional in developing embryos of the medaka (Oryzias latipes). These properties make this promoter

suitable for inducible, tissue-specific expression of transgenes and for in vivo studies of gene function and regulation. (20 ref).

7
**Effect of growth hormone on the growth rate of the gilthead seabream (Sparus aurata), and use of different constructs for the production of transgenic fish. effect of cattle, human, fowl, pig or recombinant native somatotropin on growth rate, and reporter gene expression in transgenic fish (conference paper)**
Cavari-B; Funkenstein-B; Chen-T-T; Gonzalez-Villasenor-L-I; Schartl-M *Aquaculture; (1993) 111, 1-4, 189-97* (1993).
*Descriptor:* cattle, human, fowl, pig, gilthead seabream recombinant somatotropin effect on growth rate, reporter gene expression in transgenic fish * hormone mammal bird gene transmission cloning microinjection Sparus aurata
*Abstract*: When cattle or human somatotropins were injected into 6-mth-old (about 10 g) gilthead seabream (Sparus aurata), the growth of the fish, as measured in changes in their wt., increased by only about 15% compared with a saline-injected control. No effect or slight inhibition of the growth rate was obtained when fowl or pig somatotropins were injected. Injection of the native somatotropin increased the growth rate of the fish by about 20% after treatment for 2 wk. An expression vector, using plasmid pRE1 and transformation into MZ1 cells, produced the gilthead seabream somatotropin, providing a supply for further experiments on the effect of the homologous hormone on growth. 2 Reporter genes, beta-galactosidase (lacZ, EC-3.2.1.23) and the melanoma oncogene of Xiphophorus (mrk YY), were microinjected into fertilized eggs of S. aurata using vector plasmid pCMVTklacZ and plasmid pCMVTkmrkYY, respectively. Expression of these 2 genes could be demonstrated in 2-day-old embryos, the lacZ gene by staining of its enzymatic product, and the mrk YY gene by its phenotypic expression (embryonal tumor). (18 ref).

8
**Efficient transient expression system based on square pulse electroporation and in vivo luciferase assay of fertilized fish eggs. African catfish, zebrafish and rosy barb transgenic fish breeding**
Mueller-F; Lele-Z; Varadi-L; Menczel-L; +Orban-L *FEBS-Lett.; (1993) 324, 1, 27-32* (1993).
*Descriptor:* firefly luciferase transient expression in African catfish, zebrafish, rosy barb embryo, electroporation, pot. transgenic fish breeding * insect arthropod enzyme EC-1.13.12.7 reporter gene transmission cloning plasmid pCMV; 1
*Abstract*: Fertilized eggs of African catfish (Clarias gariepinus), zebrafish (Brachydanio rerio) and rosy barb (Barbus conchonius) were dechorionated using pronase E type XXV (Sigma) and then subjected to electroporation with plasmid pCMV/1, in which a firefly luciferase (EC-1.13.12.7) gene was driven by the cytomegalo virus 1E1 promoter. Electroporation-mediated DNA transfer was improved by applying a train of square pulses. For African catfish, best results were obtained with 100 V/cm and 16 pulses with 200 usec pulse length and 500 usec pulse distances. In 1 treatment (100 V/cm, 16 pulses), 47.5% of 1-day-old living embryos (19/40) showed luciferase expression. No DNA transfer was observed when non-dechorionated eggs or exponential decay pulses were used. For zebrafish, a field strength of 80 or 100 V/cm and 16 pulses gave 20% positives at the gastrula stage, and for rosy barb, 50% positives were obtained at 100 V/cm and 24 pulses. Temporal expression patterns were very variable; embryos showed a wide variation in the onset and duration of expression. However, development of the method may allow genetic improvement of farm fish spp. (17 ref).

9 **NAL Call No.: 49-J82**
**Environmental safety issues for genetically modified animals.**
Bruggemann, E. P. *J-anim-sci* v.71, p.47-50. (1993).
Paper presented at the symposium, "Genetically Modified Livestock: Progress, Prospects, and Issues", August 11, 1992, Pittsburgh, Pennsylvania.
*Descriptor:* transgenic-animals; introduction-; livestock-; domestic-animals; fishes-; recombinant-vaccines; environmental-impact; risk-; safety-
*Abstract*: Organisms modified by the techniques of modem biotechnology may differ significantly from normal organisms or organisms modified by other methods. Before transgenic organisms are introduced into the environment, the potential environmental effects should be assessed. In

general, modification of ecologically important traits in undomesticated species presents the greatest environmental risk. Transgenic livestock probably pose low risk to the environment. Transgenic fish and live virus-based vaccines pose greater risks and present challenging questions for environmental risk assessment.

10
**Environmental safety issues for genetically modified animals. transgenic animal release in environment e.g. transgenic fish construction via somatotropin and antifreeze gene cloning and live virus e.g. rabies virus recombinant vaccine (conference paper)**
Bruggemann-E-P *J.Anim.Sci.; (1993) 71, Suppl.3, 47-50* (1993).
*Descriptor:* transgenic animal release in environment e.g. transgenic fish, live virus e.g. rabies virus recombinant vaccine * hormone somatotropin antifreeze gene cloning gene transmission
*Abstract*: Transgenic livestock probably pose low risk to the environment but transgenic fish and live virus-based vaccines pose greater risks. An agency is required that will cover all environmental introductions of transgenic organisms and that will administer the statute, write regulation and oversee all introductions. Transgenic fish may be difficult to control or eradicate due to their high reproductive capacity. Two modifications of fish are currently being developed: (1) insertion of somatotropin into the fish for increased body weight and quality (although this may lead to direct competition with the native species and result in their decline); and (2) production of antifreeze proteins to develop fish that can survive in cold water. An antirabies vaccine for wild raccoons based on vaccinia virus and an antipseudorabies vaccine for pigs based on swine-pox virus are currently being developed. Both viruses are designed to be oral vaccines that can be delivered to animals in bait distributed by air. The vaccinia virus has a broad host range and the potential for infecting species other than raccoon is possible. (28 ref).

11
**Expression of yellow tail (Seriola quinqueradiata) fish growth hormone cDNA in the marine photosynthetic bacterium Rhodobacter sp. NKPB 0021. using vector plasmid KGH319 for expression of fish recombinant somatotropin**
Burgess-J-G; Tsubaki-K; +Matsunaga-T
*Biotechnol.Lett.; (1993) 15, 2, 111-14* (1993).
*Descriptor:* yellow tail fish recombinant somatotropin prep., vector plasmid KGH319, expression in Rhodobacter sp. * Seriola quinqueradiata gene cloning transformation bacterium hormone
*Abstract*: The yellow tail (Seriola quinqueradiata) growth hormone (ytGH, somatotropin) gene was cloned into vector plasmid pUC18. The resultant construct, plasmid pUGH12, was used with shuttle vector plasmid pUK319 to create plasmid pKGH319 in which the ytGH open reading frame was located downstream of the lac promoter. Escherichia coli JM109 (pKGH319) was used as a source of plasmid DNA for Rhodobacter sp. NKPB0021 transformation, with transformants selected in the presence of kanamycin. Over 65% of colonies harboring pKGH319 maintained kanamycin selection after 200 generations in the absence of antibiotic. The presence of the ytGH gene did not decrease plasmid stability. Plasmids were maintained during photosynthetic growth and showed excellent stability compared to other broad host range plasmids used for freshwater photosynthetic bacteria, which are rapidly lost during phototrophic growth. Plasmid pUK319 was also stably maintained in E. coli JM109, showing that the presence of the plasmid pRD31 replicon did not disrupt plasmid segregation in pUK318. Immunoblot analysis indicated low level in vivo gene expression. (13 ref).

12 **NAL Call No.: QH442.6.G45-1993**
**Gene transfer and expression in farmed fish.**
Dunham, R. A. 1.; United States Israel Binational Agricultural Research and Development Fund. Bet Dagan, Israel : BARD, 1993. 57 leaves : ill.. Final report.
*Descriptors:* Fishes-Genetic-engineering; Transgenic-animals; Gene-expression

13
**Gene transfer via electroporation in fish. breeding of loach and red crucian carp transgenic fish expressing the human somatotropin gene (conference paper)**

Xie-Y; Liu-D; Zou-J; Li-G; Zhu-Z *Aquaculture; (1993) 111, 1-4, 207-13* (1993).
*Descriptor:* human recombinant somatotropin expression in loach, red crucian carp transgenic fish * gene transmission cloning protein hormone Misgurnus anguillicaudatus Carassius auratus auratus
*Abstract*: Recombinant plasmid pMhGH contained a 1968 bp fragment of the mouse metallothionein-1 promoter driving a 1500 np fragment of the human somatotropin (hST) gene, of which the last 3 of the 4 introns were removed. The construct was introduced into dechorionated eggs of loach (Misgurnus anguillicaudatus Cantor) and red crucian carp (Carassius auratus auratus Linnaeus) by electroporation. Dot blot analysis showed that the efficiency of gene transfer (GTE) and the transgene copy number per genome (CNP) increased as the pulse duration increased in the range 0.48-4.8 msec. Both the GTE and CNP increased as the voltage pulse rose. The highest GTE, 62.5%, was obtained with electric pulses under the following conditions: pulse voltage, 300 V; and pulse duration, 0.97 msec (capacitance 22 uF, 20 Ohms). CNP reached over 100. Dot-blots showed that replication of foreign DNA occurred at the blastula stage and peaked during the late gastrula stage. Alterations of electric capacity, within the range examined, and the plasmid form (circular or linear) had no obvious effect on the GTE of red crucian carp. (12 ref).

14 **NAL Call No.: QH442.6.G46--1993**
**Genetic engineering of animals.**
Puhler, A. Weinheim ; New York : VCH, c1993.
177 p. : ill. (some col.).
Includes bibliographical references and index.
Genetic engineering of animal cells / M. Wirth, H. Hauser -- Expression of endogenous and heterologous genes in animal cells -- Manipulation of animal cells -- Transgenic animals / G. Brem -- Transfer of transgenes -- Transmission of transgenes -- Expression of transgenes -- Application of transgenic mice -- Application of transgenic livestock -- Transgenic chicken -- Transgenic fish -- Problems and future possibilities with transgenic animals.
*Descriptors:* Transgenic-animals

15
**Genetic improvement of disease-resistance in fish: an overview. fish breeding through selection and transgenic fish production; a review (conference paper)**
Fjalestad-K-T; Gjedrem-T; Gjerde-B *Aquaculture; (1993) 111, 1-4, 65-74* (1993).
*Descriptor:* fish farming, improved disease-resistance, transgenic fish breeding, a review
*Abstract*: Problems involved in increasing fish disease-resistance were discussed. In a breeding program, the economically important traits included in the breeding goal must be defined and the measurement of each trait must be performed meaningfully. Disease-resistance can be measured by examination of the rate of survival, by exposing the fish to specific disease agents, or by immunological or physiological parameters. Selection may be performed both directly or indirectly, by correlated response, or by examination of expected responses to selection for increased survival. Breeding programs frequently include crossbreeding between strains or lines to utilize heterosis effects. Disease-resistance genes have not been identified in fish. Wild strains may have disease-resistance genes that could be of advantage in fish farming. Such genes may be transferred into farming populations by systematic crossing. The production of transgenic fish with enhanced resistance to specific diseases remains a possibility for the future. Gene technology research should focus on gene mapping and isolation of genes regulating disease- resistance. (50 ref).

16
**Growth hormone gene and the transgenic fish. fish farming application (conference paper)**
Zhu-Z *Biotechnol.Agric.; (1993) 145-55* (1993).
*Descriptor:* human, fish somatotropin gene appl. transgenic fish construction, fish farming * mammal growth hormone vector plasmid pCAgGH gene transmission
*Abstract*: The human somatotropin gene was used to produce the first batch of transgenic fish and establish a transgenic fish model. The transgenic fish with a functional human somatotropin gene grew 4.6 times faster than the control. This study has shown the importance of gene transfer for fish breeding. Fish somatotropin gene expression in the transgenics is little understood; a fish somatotropin gene construct will be constructed for the production of farmed transgenic species. A

transgenic fish model was presented, as was a new construct of an 'all-fish' somatotropin gene (plasmid pCAgGH) which was designed for production of farmed transgenics. A few key questions for further studies of fish transgenics were also discussed. (29 ref).

17

**Growth hormones and transgenic animals. foreign somatotropin gene expression in transgenic animal for enhanced growth rate; a review**

Palanivelu-P; Dharmalingam-K *Curr.Sci.; (1993) 64, 3, 169-75* (1993).

*Descriptor:* recombinant somatotropin expression in transgenic animal, mammal, fish, review * hormone

*Abstract*: The protein and gene structures of somatotropins (growth hormones) are discussed in relation to the expression of somatotropin genes in transgenic animals as a means of enhancing growth rate. A comparison is made of the protein sequences of human, pig, fowl, rat, goat, trout, salmon, carp and eel somatotropins. Fish and mammalian somatotropins have been successfully expressed in Escherichia coli, Streptomyces fradiae, Bacillus sp. and mammalian cells. The use of metallothionein-based vectors has been particularly useful for the expression of somatotropin cDNA in cultured mammalian cells as well as in transgenic animals. Current methods available for the breeding of transgenic animals, e.g. microinjection of genes into fertilized eggs, embryonic stem cell technology, the use of retro virus systems, microprojectile technology and electroporation are described. Although the microinjection method is widely used for production of transgenics, the method is limited by the resulting poor rate of integration, poor expression of integrated genes and the absence of hereditary transmission. However, some successful results have been reported. (41 ref).

18

**Integration and germ-line transmission of a pseudotyped retro viral vector in zebrafish. transgenic fish breeding using retro virus vector**

Lin-S; Gaiano-N; Culp-P; Burns-J-C; Friedmann-T; +Hopkins-N *Science; (1994) 265, 5172, 666-69* (1994).

*Descriptor:* zebrafish transgenic fish breeding, pseudotyped retro virus vector LZRNL, provirus integration, germ-line transmission * Danio rerio vesicular-stomatitis virus

*Abstract*: To determine if a retro viral vector pseudotyped with the envelope glycoprotein of the vesicular-stomatitis virus could infect zebrafish (Danio rerio) embryos and, in particular, the cells destined to become the germ-line, a pseudotyped virus, LZRNL, was injected into blastula- stage zebrafish embryos. Injected embryos were raised to sexual maturity and mated, and DNA from 24-hr-old pools of their F1 progeny were tested for LZRNL sequences by polymerase chain reaction. Of 51 fish examined, 8 showed germ-line transmission of the retro viral sequences. The 8 founder lines had mosaic germ-lines and transmitted proviral DNA to less than 5% of their F1 progeny. The progeny of transgenic F1 matings demonstrated Mendelian inheritance, suggesting that proviral DNA was integrated into the zebrafish genome. Transgenic F1 fish inherited a single integrated provirus, and a founder could transmit more than 1 viral integration to its progeny. The results demonstrated that this pantropic pseudotyped vector will make the use of retro virus vectors in zebrafish possible, e.g. for studies of vertebrate development. (28 ref).

19

**Liposome-mediated gene transfer in fish embryos. reporter gene expression in transgenic fish (African catfish)**

Szelei-J; Varadi-L; Mueller-F; Erdelyi-F; +Orban-L; Horvath-L *Transgenic-Res.; (1994) 3, 2, 116-19* (1994).

*Descriptor:* transgenic fish breeding, reporter gene expression in African catfish, liposome-mediated gene transmission * transgenic animal Clarias gariepinus cloning chloramphenicol-acetyltransferase enzyme EC-2.3.1.28 neomycin-phosphotransferase mouse metallothionein-I mammal tumor necrosis factor promoter lipofection

*Abstract*: Liposome-mediated gene transmission was used to introduce large DNA constructs into zygotes of African catfish (Clarias gariepinus). Recombinant phage lambda particles or DNA-protamine complexes were delivered into the cytoplasm of target cells by negatively-charged, large unilamellar liposomes. Phage lambda constructs contained: the chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) gene under control of the mouse

Friend-leukemia virus long terminal repeat promoter; neomycin-phosphotransferase (neo) gene under control of the Rous-sarcoma virus (RSV) enhancer and the thymidine-kinase (TK) promoter of herpes simplex virus-I; the mouse metallothionein-I (mMTHI) gene and promoter; or the human tumor necrosis factor gene regulated by the SV40 virus early promoter. Plasmid pNMSV-2, used to produce plasmid- containing liposomes, contained the promoter/gene combinations SV40/CAT, mMTHI/mMTHI or RSV-tk/neo. Dechorionated zygotes and early embryos were treated with the liposomes. Expression of the introduced reporter genes was followed during the first 3 wk of the development of the larvae. Very efficient DNA uptake into the embryos was indicated. (23 ref).

20

**Lymphocyte expression in transgenic trout by mouse immunoglobulin promoter-enhancer. rainbow trout transgenic fish breeding to examine the fish immune system**

Michard-Vanhee-C; Chourrout-D; Stromberg-S; Thuvander-A; +Pilstrom-L *Immunogenetics; (1994) 40, 1, 1-8* (1994).

*Descriptor:* mouse immunoglobulin promoter-enhancer-mediated tissue-specific gene expression, rainbow trout transgenic fish * Oncorhynchus mykiss chloramphenicol-acetyltransferase enzyme EC-2.3.1.28 reporter gene cloning antibody

*Abstract*: 2 Groups of transgenic rainbow trout (Oncorhynchus mykiss, Walbaum) were produced. 1 Group harbored the chloramphenicol-acetyltransferase (CAT, EC-2.3.1.28) reporter gene associated with mouse immunoglobulin (Ig) promoter/enhancer (plasmid pUCL-CAT-E). The 2nd group carried the CAT gene under the control of the cytomegalo virus promoter/enhancer (plasmid pCMV-CAT). Slot-blot analysis of DNA from pUCL-CAT-E fish showed variation of copy number between the major tissues but not between red (RBC) and white blood cells (WBC). Southern blotting indicated that multiple copies organized in concatamers were incorporated into the genome. The pCMV-CAT fish expressed CAT in WBC and RBC. pUCL-CAT-E fish expressed CAT in WBC but not in RBC. Expression in WBC was found preferentially in sIg+ cells, suggesting that B-lymphocytes are the major expressors. High expression was also found in spleen and kidney. High WBC expression was associated with low tissue expression, except that liver (containing lymphoid tissue in fish) was higher. Thus, the regulatory elements of the Ig gene from mouse induced tissue-specific expression in fish. (36 ref).

21

**Marine biotechnology in Singapore. application of aquaculture**

Meng-L-T; Lee-C-K *Australas.Biotechnol.; (1994) 4, 4, 226-28* (1994).

*Descriptor:* aquaculture, marine biotechnology, Singapore * (Vol.13, No.23)

*Abstract*: The following recent developments and current projects in marine biotechnology and related activities in Singapore were discussed: (1) extraction and identification of bioactive compounds from coral reef organisms; (2) isolation of antibiotics in horseshoe crabs; (3) biochemical and pharmacological studies on toxins of marine organisms such as the Thunder Crab (Lyphozozymus pictor) and the stone-fish (Synanceja horrida); (4) transgenic fish production using e.g. the Marby Goby (Oxyeleotris marmorata, Bleeker), grouper and salmon; (5) purification of fish somatotropin and cloning of their genes in the Zebrafish (Japanese medaka) experimental model; (6) fish breeding and fry production using e.g. thyroid hormones in enhancing embryo and fry development; (7) DNA fingerprinting of aquarium fishes to allow genetically compatible fish to cross-breed for better quality offspring; and (8) other aquaculture-related research including development of vaccines and antibodies. (0 ref).

22

**Micromachined electroporation system for transgenic fish. medaka fertilized egg transformation**

Murakami-Y; Motohashi-K; Yano-K; Ikebukuro-K; Yokoyama-K; +Karube-I *J.Biotechnol.; (1994) 34, 1, 35-42* (1994).

*Descriptor:* medaka transgenic fish construction, fertilized egg transformation, micromachined electroporation, firefly luciferase reporter gene, vector plasmid pRSVL * transgenic animal Oryzias latipes cloning enzyme EC-1.13.12.7 insect arthropod Photinus pyralis

*Abstract*: A micromachined electroporation system for transgenic fish construction is described. The

attaching filaments were removed from fertilized eggs of medaka before cleavage and the eggs were rinsed twice with distilled water and then soaked in mannitol buffer. The device fabricated was sterilized with ethanol and filled with 40 ul of a solution of plasmid pRSVL DNA encoding firefly (Photinus pyralis) luciferase (EC-1.13.12.7). The egg was placed between the electrodes and an electric pulse was created with a gene introduction instrument (GTE-1) to introduce the luciferase gene into the egg cell. The pulse conditions were 30 V for 50 usec repeated 5 times with 1 sec intervals. The eggs were then rinsed twice with distilled water and cultured at 26 deg in distilled water for 7 days. The new method is simpler than microinjection and introduces DNA at an introduction rate of 26% into either end of an egg cell. By localizing introduction to the poles of the eggs, egg yolk destruction is avoided. However, correct egg cell positioning for this localization limits mass handling. (21 ref).

23

**Molecular cloning and expression in Escherichia coli of the glycoprotein gene of VHS virus, and immunization of rainbow trout with the recombinant protein.**
**viral-hemorrhagic-septicemia virus recombinant vaccine construction for fish protection; Factor-Xa fusion protein with virus glycoprotein and fusion protein cleavage**
Lorenzen-N; Olesen-N-J; Vestergard-Jorgensen-P-E; Etzerodt-M; Holtet-T-L; Thogersen-H-C
*J.Gen.Virol.; (1993) 74, Pt.4, 623-30* (1993).
*Descriptor:* viral-hemorrhagic-septicemia virus envelope glycoprotein gene cloning, expression as Factor-Xa fusion protein in Escherichia coli, pot. fish e.g. rainbow trout recombinant vaccine, DNA sequence * bacterium salmonid rhabdo virus blood-clotting protein sequence fusion protein cleavage
*Abstract*: A gene encoding the envelope glycoprotein of a Danish isolate of a salmonid rhabdo virus (sRV), viral-hemorrhagic-septicemia virus (VHSV) was cloned and sequenced. When compared with the deduced sequence of a French isolate of VHSV, 13 amino acid substitutions were noticed in the Danish virus. Amino acid homologies with the glycoprotein of a North American sRV (infectious-hematopoietic-necrosis virus) indicated a high degree (38%) of structural similarity between the 2 fish sRV glycoproteins. The Danish glycoprotein gene comprised 1521 nucleotides encoding a protein of 507 amino acid residues (mol.wt. 57,000). The glycoprotein lacking the N-terminal leader sequence and C-terminal hydrophobic anchor segment was expressed in Escherichia coli as a Factor-Xa protease-cleavable fusion protein on transformation with expression vector plasmid pCMHVG-1. The purified and renatured viral part of the fusion protein elicited VHSV-specific antibodies and neutralizing antibody activity when injected into rainbow trout. The recombinant protein may be useful as a recombinant vaccine. (31 ref).

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**Molecular cloning and nucleotide sequence analysis of the maltose-inducible porin gene of Aeromonas salmonicida. as a preliminary study of the potential use of the porin as an immunogen in furunculosis vaccine development**
Dodsworth-S-J; Bennett-A-J; +Coleman-G
*FEMS-Microbiol.Lett.; (1993) 112, 2, 191-98* (1993).
*Descriptor:* Aeromonas salmonicida maltose-inducible porin gene cloning, DNA sequence, pot. fish furunculosis vaccine development * bacterium
*Abstract*: The Aeromonas salmonicida maltose-inducible porin (maltoporin) is potentially useful as an immunogen in the development of a vaccine to protect salmonid fish against furunculosis. As part of a preliminary investigation, the gene encoding the maltoporin of non-pathogenic A. salmonicida Unilever 2862 was cloned into phagemid pTZ18R in 2 restriction fragments, 0.6 kb Pst/KpnI and 1.7 kb SphI, of genomic DNA and their DNA sequences were determined. Open reading frames of 1329 and 1335 bp translated into sequences of 443 and 445 amino acids, with a 23 or 25 amino acid signal peptide sequence and a 420 amino acid mature protein of mol.wt. 46,424. Putative ribosome binding sites, AGGA and GGGAA, occurred 9 bp upstream of 2 possible ATG initiation codons. The A. salmonicida gene product showed a high degree of similarity with Escherichia coli porin LamB, and codon usage was very similar to that of another A. salmonicida outer membrane protein but markedly different from those of extracellular proteins. (12 ref).

25
**New developments in marine biotechnology. military marine biotechnology and genetic engineering (conference paper)**
Colwell-R-R *Biotechnol.Aerospace-Appl.; (1989) 99-109* (1989).
*Descriptor:* military space marine biotechnology new developments e.g. genetic engineering of fish
*Abstract*: Some of the dynamically developing areas of marine biotechnology, genetic engineering of fish and the exploration of marine bioactive metabolites, exemplify the potential of this new field. Aplysina fistualaris produces brominated aromatic compounds with antibiotic and adrenergic activity. Dasychalina cyathina produces nucleosides which have asystolic and cardiovascular activities. Halichondaria okadai produces okadaic acid and ionophores with cytostatic and antibiotic activities. Luffariella variabilis produces monoalides with antibiotic and antiinflammatory activities. Pandaros acanthifolium produces acanthifolicin antibiotics and Siphonodictyon coralliphagum produces siphondictyal compounds with antibiotic and calcium chelating activities. The possibility of increasing harvests of seafood by more cost-effective methods will be offered by marine biotechnology. Marine biotechnology applications may include DNA probes for detecting marine pathogens, genetically engineering disease-resistance, detoxification of industrial chemicals, genetic production of salt-tolerant plants, marine bioelectronics and signaling and antifouling. (16 ref).

26
**One-armed PCR: a novel method for amplification of genomic DNA. one-armed polymerase chain reaction for e.g. Japanese puffer fish genome DNA amplification (conference abstract)**
Macrae-A; McHale-M; Elgar-G; Brenner-S *Genome-Mapping-and-Sequencing; (1993) 150* (1993).
*Descriptor:* one-armed polymerase chain reaction method, appl. e.g. Japanese puffer fish genome DNA amplification from single known sequence area * animal cloning Fugu
*Abstract*: The small vertebrate genome of the Japanese puffer fish Fugu was used to develop a novel method, one-armed polymerase chain reaction (PCR), to allow amplification of genomic DNA from a single area of known sequence. A small insert Fugu genomic gene bank was generated for amplification from a phage vector-specific DNA primer to the area of interest. 2-10 kb DNA fragments were size selected and cloned in phage MGU2. DNA from this gene bank was used as a PCR template. PCR was performed between an area of known sequence and a tailed vector-specific primer. An aliquot of this reaction was used as a template for a 2nd round of nested PCR with a primer homologous to the tail of the vector primer, and a 2nd sequence-specific primer. The method was used to amplify single discrete products of 70-19,000 bp (mean 600 bp), and to walk into the 5'-noncoding sequence of the Fugu dopamine-D1 receptor equivalent. The system could be used with degenerate primers to allow amplification of novel sequences. A Fugu neurokinin receptor gene was amplified, with a novel intron whose position precluded amplification by conventional PCR methods. (0 ref).

27 **NAL Call No.: QH301.N32**
**Potential impacts of transgenics and genetically manipulated fish on natural populations: addressing the uncertainties through field testing.**
Hallerman, E.; Kapuscinski, A.
*NATO-ASI-ser,-Ser-A,-Life-sci. New York : Plenum, 1984-. 1993. v. 248 p. 93-112.*
In the series analytic: Genetic conservation of Salmonid fishes / edited by J.G. Cloud and G.H. Thorgaard.
*Descriptor:* salmonidae-; transgenic-animals; genetic-engineering; environmental-impact; population-genetics; introduced-species

28
**Problems and prospects of transgenic fish production. a review**
Pandian-T-J; Marian-L-A *Curr.Sci.; (1994) 66, 9, 635-49* (1994).
*Descriptor:* transgenic fish breeding, germplasm preservation, biological containment, review * gene transmission cloning
*Abstract*: The restricted scope of cytoplasmic introduction of transgenes, and the limited availability of fish cDNA sequences and promoters, are major hurdles in the production of transgenic fish. Long-term research is required to produce drought-resistant (aestivating) transgenic carp. A

DNA concentration of 10-15 ug/ml containing 1-2 million copes of transgene is usually injected into the fish egg at its 1-cell stage. Catfish and tilapias are sensitive to microinjection, so that alternative methods of gene introduction, such as electroporation and sperm-mediated transfer, are required. A common observation is the mosaicism in transgenic fish; the delayed delivery and/or integration of the transgene may be the causative factor. Viral and metallothionein promoters are giving way to fish promoter genes, and all-fish constructs comprising endogenous fish promoters (e.g. beta-actin, AFP) have been shown to be effective. The use of sterile triploid eggs is recommended for the biological containment of transgenic fish. Sperm cryopreservation and androgenesis are required for the germplasm preservation of many Asian fish spp. (130 ref).

29 **NAL Call No.: QH301.N32**
**The reproductive containment of genetically altered salmonids.**
Donaldson, E. M.; Devlin, R. H.; Solar, I. I. *NATO-ASI-ser,-Ser-A,-Life-sci. New York : Plenum, 1984-. 1993. v. 248 p. 113-129.*
In the series analytic: Genetic conservation of Salmonid fishes / edited by J.G. Cloud and G.H. Thorgaard.
*Descriptor:* salmonidae-; sterilization-; infertility-; transgenic-animals; genetic-engineering; sexual-reproduction; environmental-impact

30 **NAL Call No.: TP248.25.A96T68-1990**
**Transgenesis of animals.**
Kondoh, H.; Agata, K.; Ozato, K. *Automation in biotechnology a collection of contributions presented at the Fourth Toyota Conference, Aichi, Japan, 21-24 October 1990 / edited by Isao Karube. Amsterdam : Elsevier c1991.. p. 203-216.*
Includes references.
*Descriptor:* mice-; fowls-; atheriniformes-; gene-transfer; transgenics-; genetic-transformation; blood-cells; reporter-genes; beta-galactosidase-; ova-; gene-expression; transcription-; embryonic-stem-cells; miwz-gene; oryzias-
*Abstract*: We discuss the principles of transgenesis, describe a model gene suitable for analysis of the process of gene transfer, and compare methodologies employed in the mouse, the chicken and the medaka fish. We also discuss embryonic stem cells which will be the major target of gene manipulation in transgenic technology in the very near future.

31
**Transgenic fish - gene transfer to increase disease and cold resistance. methods of gene transmission and transgenic fish breeding for improved disease-resistance, cold tolerance and growth promotion; a review (conference paper)**
Jiang-Y *Aquaculture; (1993) 111, 1-4, 31-40* (1993).
*Descriptor:* transgenic fish development, gene transmission methods, improved disease-resistance, cold tolerance, growth promotion, etc., review * somatotropin hormone
*Abstract*: Progress made in introducing foreign genes into fish was reviewed. The following topics were covered: (1) methods of gene transmission ((a) microinjection, (b) retro virus infection, (c) electroporation, (d) chromosome-mediated gene transfer, and (e) 'piggybacking' using sperm cells as vectors); (2) the choice of gene for fish transgenics ((a) growth promotion, by expression of a mammalian or fish somatotropin gene under the control of e.g. a metallothionein promoter in transgenic goldfish, loach, silver carp and mirror carp, etc., (b) low temp. resistance, by expressing the antifreeze proteins of e.g. winter flounder in transgenic Atlantic salmon, (c) disease-resistance, using antisense RNA constructs or the production of vaccines for fish viral disease such as infectious-hematopoietic-necrosis virus); and (3) studies on transgenic fish in China. (42 ref).

32 **NAL Call No.: aZ5076.A1U54-no.117**
**Transgenic fish research : a bibliography : a selected bibliography of research in the field of molecular biology and genetic engineering using fresh water fish.**
Warmbrodt, R. D.; Stone, V.; National Agricultural Library (U.S.). Beltsville, Md. : National Agricultural Library, 1993. viii, 48 p..
Shipping list no.: 93-0481-P.
*Descriptors:* Transgenic-fish-Bibliography

33 **NAL Call No.: QH442.B5**
**Transgenic fish: safe to eat? A look at the safety considerations regarding food transgenics.**

Berkowitz, D. B.; Kryspin Sorensen, I.
*Bio/technology-Nat-Publ-Co* v.12, p.247-252. (1994).
Includes references.
*Descriptor:* fish-; transgenic-animals; food-safety; recombinant-dna

34 **NAL Call No.: SF55.A78A7**
**Transgenic livestock.**
Jin, D. I.; Petters, R. M.; Im, K. S.
*Asian-australas-j-anim-sci* v.7, p. 1-17. (1994).
Includes references.
*Descriptor:* livestock-; transgenic-animals; gene-transfer; gene-expression; genetic-vectors; embryonic-stem-cells; liposomes-; electroporation-; genetic- transformation; performance-; milk-composition; pharmaceutical-proteins; poultry-; fishes-; somatotropin-; literature-reviews; microinjection-

35 **NAL Call No.: 100-M668; LNSU Journal-Shelves**
**Will altered fish alter environment.**
Shepard, J. *Minn-sci* v.47, p.3. ([1992?]).
*Descriptor:* fishery-resources; transgenics-; aquatic-environment; environmental-management